

gene may also lead to Parkinson's Disease (PD), other mutations are also included. In addition, it is conceivable that mutations in the related beta (46) (SEQ ID NO:11) and gamma (SEQ ID NOS: 12 and 13) may also lead to PD. Thus, mutated homologues of the alpha synuclein gene are also included in the present invention. Vectors comprising the isolated nucleic acid and host cells comprising such vectors are included as well.--

On page 10, please replace the paragraph beginning on line 7 with the following:

--The amplifying step of the present invention may be performed using the polymerase chain reaction (PCR), reverse transcriptase PCR (RTPCR), or any other type of PCR reaction known in the art. Accordingly, such a step will comprise at least one annealing step whereby at least one oligonucleotide is annealed to said sample of nucleic acids. In particular, said amplifying step uses two oligonucleotides. And in particular, the two oligonucleotides have the sequences given in SEQ ID NOS: 2 and 3.--

On page 12, please replace the paragraph beginning on line 10 with the following:
--DNA sequence of the PCR product used for mutation detection (SEQ ID NO:1)
Oligonucleotide primers are shown by arrows and the numerals 3 and 13 (SEQ ID NOS:2 and 3). Intron sequence is shown in lower case and exon sequence in upper case. Amino acid translation of the exon is shown below the DNA sequence. The circled base represents the G209A change in the mutant allele. The resulting amino acid Ala53Thr change is represented by the circled amino acid. The newly created Tsp45 I site is indicated above the DNA sequence.--

On page 13, please replace the paragraph beginning on line 9 with the following:

--Sequence alignments of alpha synuclein homologues in different species. Accession
numbers for the sequences used were as follows: Homo sapiens Swiss-Prot P37377 (SEQ ID NO:5), Bos taurus Swiss-Prot P33567 (SEQ ID NO:6), Serinus canaria genbanc L33860
(SEQ ID NO:7), Torpedo californica Swiss-Prot P37379 (SEQ ID NO:8). Numbering on top of the alignments is according to the human sequence. Amino acid 53, which is the site of the Ala53Thr change, is circled.--

On page 15, please replace the paragraph beginning on line 1 with the following: --Sequence of exons 1-7 of the human alpha synuclein gene, plus some flanking intronic sequence for each exon. (SEQ ID NOS: 14-19).--

On page 21, please replace the paragraph beginning on line 3 with the following:

--Suitable ranges of stringency conditions are described in Sambrook et al. (13).

Hybridization conditions, depending on the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5X to 0.1X SSC. Highly stringent hybridization conditions may include temperatures as low as 40°C-42°C (when denaturants such as formamide are included) or up to 60°C-65°C in ionic strengths as low as 0.1X SSC. These ranges are, however, only illustrative and, depending on the nature of the target sequence, and possible future technological developments, may be more stringent than necessary. Appropriate conditions may be determined for each specific nucleic acid sequence or oligonucleotide probe using standard control and a level of experimentation that is not considered to be undue by those of skill in the art.--

On page 26, please replace the paragraph beginning on line 15 with the following:

--In general, primers for PCR are usually about 20 bp in length, and are most preferably 15-25 bp. Denaturation of strands usually takes place at 94°C and extension from the primers is usually at 72°C. The annealing temperature varies according to the sequence under investigation. Examples of reaction times are: 20 mins denaturing; 35 cycles of 2 min, 1 min, 1 min for annealing, extension and denaturation; and finally a 5 min extension step.--

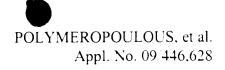
Please replace the two paragraphs beginning at page 33, line 26 with the following:

--For mutation analysis genomic DNA was amplified with oligonucleotides (3): 5'

GCTAATCAGCAATTTAAGGCTAG 3' (SEQ ID NO:2) and (13): 5' gatatgttcttagatgctcag

(SEQ ID NO:3) of genbank ID: U46898, under standard PCR conditions. Sequence analysis was performed using the Perkin Elmer dye terminator cycle sequencing kit on an ABI 373

fluorescent sequencer (ABI, Foster City, CA). Restriction digestion was performed following



the PCR with *Tsp*45 I according to manufacturer's protocol (New England Biolabs, Beverly, MA). The digested PCR products were electrophoresed on a 6% Visigel (Stratagene, La Jolla, CA), and visualized by ethidium bromide staining. Pedigree structure in Figure 2 has been slightly modified in order to protect patient confidentiality.

Total RNA was extracted from the lymphoblastoid cell line of an affected individual and first strand synthesis was performed by oligo dT priming (Gibco BRL, Gaithersburg, MD). Primers (1F) 5' ACGACAGTGTGGTGTAAAGG 3' (SEQ ID NO:9) and (13R) 5' aacatetgteageagatete 3' (SEQ ID NO:10) corresponding to nucleotides 21-40 and 520-501 of genbank L08850 were used to amplify a product of 500 bp containing the mutation at nucleotide 209. PCR products were subjected to restriction digestion by Tsp45 I. The mutation at nt 209 creates a novel Tsp45 I site (Figure 1), so that the normal allele will be restricted in 4 fragments of 249, 218, 24 and 9 bp, where the mutant allele will have 5 fragments of 249, 185, 33, 24 and 9 bp of size, as shown in Figure 3. Size standards used, were the 100 bp ladder (Gibco BRL, Gaithersburg, MD).—

A marked up copy of the amended paragraphs is included in the Appendix.